29. (NEW) The method of claim 19 wherein the second luminescently labeled reporter molecule is neuron-specific.

30. (NEW) The method of claim 29 wherein the neuron-specific luminescent reporter molecule comprises a molecule selected from the group consisting of neurofilament proteins, βIII-tubulin, ciliary neurotrophic factor, and antibodies specific for neurofilament proteins.

SUPPORT FOR THE AMENDMENTS

The new claims are fully supported in the application as filed and do not constitute new matter. The claim language is supported in Example 10, page 67 line 26 to page 88 line 3. In particular, new claims 19-22 are supported, for example, on page 74 lines 13 to page 76 line 3; and page 79 line 1 to page 82 line 13.

Claims 23 and 27 are supported throughout the application, for example, on page 70 lines 9-11.

Claims 24-26 are supported, for example, on page 73 line 11 to page 74 line 11.

Claims 28-30 are supported for example, on page 71 line 9 to page 73 line 7, as well as the originally filed claims.

Thus, the new claims do not represent new matter.

REMARKS

1. Priority

The Examiner objected to the specification, based on the assertion that it does not contain a reference to U.S. Application Serial No. 08/810,983 filed 2/27/97, now U.S. Patent No. 5,989,835 in the first sentence of the specification. The Applicants traverse this assertion. The Applicants have not claimed priority to the cited co-pending application, and thus have not included it in the first sentence of the specification (see the Preliminary Amendment filed November 20, 2000). Therefore, the Applicants respectfully request reconsideration and withdrawal of this objection.

2. Information Disclosure Statement

The Applicants will submit an information disclosure statement under separate cover.

3. Drawings

The Examiner has indicated that the drawings in this case were objected to by the Draftsperson as informal. However, the Applicants were not provided with any documentation from the Draftsperson as to what, if any, informalities need to be corrected. In fact, the Applicant submitted formal drawings with the application filing. Therefore, the Applicants respectfully request withdrawal of this objection, or a notice from the Draftsperson as to the alleged informalities in the drawings, so that the Applicants can effect corrections to the drawings.

4. Oath/Declaration

The Examiner asserts that a replacement oath/declaration is required to (I) the declaration is directed to application number 09/632,552 filed August 4, 2000 but the instant application is for U.S. Application 09/716,732 filed November 20, 2000; and (II) Because no zip code is provided for the third inventor.

(I) The present application, U.S. Application 09/716,732 filed November 20, 2000, is a continuation of U.S. Application for Patent S/N 09/632,552, filed August 4, 2000. According to MPEP 602.05 "A continuation or divisional application filed under 37CFR 1.53(b) (other than a CIP) **may be filed with a copy of the oath or declaration from the prior nonprovisional application.**" (citing 37 CFR 1.63(d)(1)(iv)). Thus, there is no requirement that the Applicants file a substitute declaration in the present case, since the instant application was filed under 1.53(b) as a continuation of U.S. Application for Patent S/N 09/632,552, filed August 4, 2000, and thus can use a copy of the declaration from that case.

(II) The Applicant has not pointed to any authority that states a requirement for a ZIP code for a foreign inventor. The declaration provides an address for the third inventor, at which mail can be sent to him, which fulfills the requirement. Thus, the Applicants respectfully requests that the Examiner indicate the authority that requires the Applicants to prepare a new declaration based on the lack of a ZIP code for a foreign inventor.

Based on the above, the Applicants respectfully request reconsideration and withdrawal of this rejection.

5. Specification

a) The Applicants are herewith submitting a substitute specification with 1 ½ line

spacing throughout, as requested by the Examiner.

b) The Applicants have amended the specification to reflect the issuance of U.S. Patent No. 6,103,479.

c) The Applicants have amended the specification as requested by the Examiner to capitalize trademarks where they appear.

6. Claim Objections

(a) The Examiner objected to claim 1 based on the assertion that no punctuation followed the term "comprising". The Examiner recites MPEP608.01(m) for the proposition that a colon or semi-colon should be added after "comprising." However, the cited MPEP section says nothing about a requirement for punctuation after "comprising", nor do any of the cases cited by the Examiner. Thus, the Applicants traverse this objection on the basis that the Examiner has not pointed to any authority that requires the use of a colon or semi-colon following "comprising." However, solely in order to expedite prosecution of the present invention, and without prejudice to their refiling in a subsequent continuation, the Applicants have canceled the claims, thus obviating their rejection. Therefore, the Applicants respectfully request reconsideration and withdrawal of this objection.

(b) The Examiner objected to claim 1 as using both "luminescently labeled" and luminescently-labeled", based on the assertion of a need for consistency, and also to "clarify that the claim is directed to only one label and not two". The Applicants traverse this rejection.

One of skill in the art would have been aware that both uses were for the purpose of modifying "the macromolecule". However, solely in order to expedite prosecution of the present invention, and without prejudice to their refiling in a subsequent continuation, the Applicants have canceled the claims, thus obviating their rejection. Therefore, the Applicants respectfully request reconsideration and withdrawal of this objection.

(c) The Examiner objected to claim 18 under 37 CFR1.75(c) as being of improper dependent form, based on the assertion that the claim does not further limit the subject matter of a previous claim. The Applicants traverse this rejection.

According to MPEP 608.01(n)(III), "The test as to whether a claim is a proper dependent claim is that it shall include every limitation of the claim from which it depends." Claim 18 recites that the computer readable medium contains a program for causing a cell screening

system to execute the method of claims 1, and limits the type of cell screening system; thus under the analysis taught by the MPEP, it is a proper dependent claim. Furthermore, it is standard claim language found in many issued U.S. patents, and thus has not been found to be improper by the U.S. PTO.

However, solely in order to expedite prosecution of the present invention, and without prejudice to their refiling in a subsequent continuation, the Applicants have canceled the claims, thus obviating their rejection. Therefore, the Applicants respectfully request reconsideration and withdrawal of this objection.

7. Rejections under 35 USC Section 112, second paragraph

(a) Claim 1 was rejected based on the assertion that one of skill in the art would not know what is encompassed by "neurite outgrowth". The Applicants traverse this rejection. As admitted by the Examiner, the disclosure defines the term "neurite outgrowth" on page 69 lines 1-3. The claims are read in light of the specification in such a case (See MPEP 2111.01). Thus, it is standard patent law that the claims are read in light of the specification; indeed, that is the entire point of the specification. Applying the Examiner's logic, any term could be considered "relative", and thus the entire specification would need to be inserted into the claims. One of skill in the art would clearly understand the scope of the claim, since the claims are read in light of the specification, and the specification defines the term (as admitted by the Examiner), that the Examiner asserts to be indefinite.

Despite the above, the pending claims have been canceled, thus obviating the rejection. Therefore, the Applicants respectfully request reconsideration and withdrawal of this rejection.

(b) Claim 1 was further rejected based on the assertion that the term "valid internalized cell surface receptor" is not defined by the claims. The Applicants traverse this objection. The Applicants respectfully point out to the Examiner that there is nothing in the pending claims regarding cell surface receptor internalization. As a result, the Applicants cannot adequately respond to the rejection, as it is unclear whether the rejection is an inadvertent typographical error relating to a different case, or if the rejection is actual, and the Examiner's statements are inadvertent.

In any event, the Applicants have canceled the claims to obviate the rejection. Thus, the Applicants respectfully request reconsideration and withdrawal of this rejection.

(c) Claim 1 was rejected as being indefinite based on the assertion that the phrase "automatically determine" is indefinite, and that the claim is directed towards an automated

method for evaluating cell surface receptor internalization in a cell. The Applicants traverse this rejection. The Applicants again respectfully point out to the Examiner that there is nothing in the pending claims regarding cell surface receptor internalization. As a result, the Applicants cannot adequately respond to the rejection, as it is unclear whether the rejection is an inadvertent typographical error relating to a different case, or if the rejection is actual, and the Examiner's statements are inadvertent.

In any event, the Applicants have canceled the claims to obviate the rejection. Thus, the Applicants respectfully request reconsideration and withdrawal of this rejection.

(d) Claim 1 was rejected as being indefinite based on the assertion that essential steps were omitted. Specifically, the Examiner asserted that the claims do not recite a separation step to eliminate bound and unbound materials, which the examiner asserts is a critical step in the method. The Applicants traverse this rejection.

A separation step is not a requirement for practicing the methods of the invention. In a non-limiting example, if the cell expresses a recombinant cell surface receptor-green fluorescent protein chimera, then no exogenous luminescence is present, and no separation step is required. Thus, no essential step was omitted. However, solely in order to expedite prosecution of the application, the Applicants have canceled the claims to obviate the rejection. Thus, the Applicants respectfully request reconsideration and withdrawal of this objection.

8. Double patenting rejections

(a) The Examiner provisionally rejected claims 1 and 18 for obviousness type double patenting over claims 52-57 and 70-91 of co-pending Application No. 09/398,965. The Applicants traverse this rejection. The claims in the cited co-pending application relate to analyzing microtubule structure. However, solely in order to expedite prosecution of the application, the Applicants have canceled the claims to obviate the rejection. Thus, the Applicants respectfully request reconsideration and withdrawal of this rejection.

(b) The Examiner provisionally rejected claims 1 and 18 for obviousness type double patenting over claims 1 and 40-48 of co-pending Application No. 09/352,171. The Examiner asserts that "Neuronal cell internalization via the same methods reads on the same inventive scope" of the 09/352,171, which recites methods for analyzing cell surface receptor protein internalization." The Examiner further asserts that "It would have been obvious to the skilled practitioner in the art to employ various other cell surface proteins, such as those found in neuron

cellular analysis, as they would relate to cellular evaluation in the same automated method." The Applicants traverse this rejection.

The Applicants do not understand the Examiner's assertion recited above, and are not certain whether portions of this rejection are inadvertent, based on the various inadvertent statements by the Examiner that the present claims recite methods for cell surface receptor internalization. Thus, the Applicants cannot comment specifically on this rejection. However, solely in order to expedite prosecution of the application, the Applicants have canceled the claims to obviate the rejection. Thus, the Applicants respectfully request reconsideration and withdrawal of this rejection.

9. Rejections under 35 USC 103(a)

(a) Claims 1 and 18 were rejected as being obvious over Taylor et al. (Optical Diagnostics) or Taylor et al. (American Scientist) in view of Lee et al. and Schroeder et al., based on the assertion that the combination of these references would make the instantly claimed methods obvious to one of skill in the art. The Applicants traverse this rejection.

In order to establish a *prima facie* case of obviousness the Examiner must establish three criteria; 1) a suggestion or motivation found within the prior art or within the knowledge of one of skill in the art to combine or modify the references; 2) a reasonable expectation of success; and 3) the prior art references alone or in combination must teach or suggest *all* the claim limitations. MPEP § 706.02(j).

Presently pending claim 19 recites an automated method for analyzing neurite outgrowth comprising

a) providing an array of locations comprising cells, wherein the cells possess at least a first luminescently labeled reporter molecule that reports on cell location, and at least a second luminescently labeled reporter molecule that reports on neurite outgrowth;

b) obtaining a nuclear image from the at least first luminescently labeled reporter molecule and a neurite image from the at least second luminescently-labeled reporter molecule;

c) automatically identifying cell bodies from the nuclear image;

d) automatically identifying neurites extending from the cell bodies from the neurite image; and

e) automatically determining one or more neurite features selected from the group consisting of:

i) Total neurite length from all cells;

ii) Total number of neurite branches from all cells;

iii) Number of neurites per cell;

iv) Number of neurites per positive neuron;

v) Neurite length from each cell;

vi) Neurite length per positive neuron;

vii) Neurite length per neurite;

viii) Number of cells that are positive for neurite outgrowth;

ix) Percentage of cells positive for neurite outgrowth;

x) Number of branches per neuron; and

xi) Number of branches per neurite.

The Taylor references teach methods for automated image acquisition using fluorescent reporter molecules, and does not teach any methods for automated data analysis, and do not teach anything regarding analyzing neurite extension. Specifically, neither of the Taylor references teach or suggest any of the following limitations from presently pending claim 19:

a) providing an array of locations comprising cells, wherein the cells possess at least a first luminescently labeled reporter molecule that reports on **cell location**, **and** at least a second luminescently labeled reporter molecule that **reports on neurite outgrowth**;

b) obtaining a **nuclear image** from the at least first luminescently labeled reporter molecule **and a neurite image** from the at least second luminescently-labeled reporter molecule;

c) automatically **identifying cell bodies** from the nuclear image;

d) automatically **identifying neurites** extending from the cell bodies from the neurite image; and

e) automatically determining one or more neurite features selected from the group consisting of:

i) Total neurite length from all cells;

ii) Total number of neurite branches from all cells;

iii) Number of neurites per cell;

iv) Number of neurites per positive neuron;

v) Neurite length from each cell;

vi) Neurite length per positive neuron;

vii) Neurite length per neurite;

viii) Number of cells that are positive for neurite outgrowth;

ix) Percentage of cells positive for neurite outgrowth;

x) Number of branches per neuron; and

xi) Number of branches per neurite.

The Lee reference teaches phosphorylation of kinesin, and provides no teaching or disclosure on any type of image acquisition or analysis. The reference simply teaches adding nerve growth factor to PC12 cells and determining its effect on kinesin phosphorylation. Thus, the Lee reference does not teach any of the following limitations from pending claim 19:

a) providing an array of locations comprising cells, wherein the cells possess at least a first luminescently labeled reporter molecule that reports on cell location, and at least a second luminescently labeled reporter molecule that reports on neurite outgrowth;

b) obtaining a nuclear image from the at least first luminescently labeled reporter molecule and a neurite image from the at least second luminescently-labeled reporter molecule;

c) automatically identifying cell bodies from the nuclear image;

d) automatically identifying neurites extending from the cell bodies from the neurite image; and

e) automatically determining one or more neurite features selected from the group consisting of:

i) Total neurite length from all cells;

ii) Total number of neurite branches from all cells;

iii) Number of neurites per cell;

iv) Number of neurites per positive neuron;

v) Neurite length from each cell;

vi) Neurite length per positive neuron;

vii) Neurite length per neurite;

viii) Number of cells that are positive for neurite outgrowth;

ix) Percentage of cells positive for neurite outgrowth;

x) Number of branches per neuron; and

xi) Number of branches per neurite.

Therefore, Lee does not cure any deficiencies in the Taylor references, and the combination of these references clearly does not teach or suggest the limitations of claim 19.

Finally, the Schroeder reference specifically addresses the problem of minimizing background fluorescence in obtaining fluorescence from a cell layer, by manipulating the angular relationship between the source of illumination and the detector (see in particular column 3, lines 28-31 and column 4 lines 12-18). Schroeder teaches nothing about neurite outgrowth, and does not teach or suggest any of the following limitations of claim 19:

a) providing an array of locations comprising cells, wherein the cells possess at least a first luminescently labeled reporter molecule that reports on cell location, and at least a second luminescently labeled reporter molecule that reports on neurite outgrowth;

b) obtaining a nuclear image from the at least first luminescently labeled reporter molecule and a neurite image from the at least second luminescently-labeled reporter molecule;

c) automatically identifying cell bodies from the nuclear image;

d) automatically identifying neurites extending from the cell bodies from the neurite image; and

e) automatically determining one or more neurite features selected from the group consisting of:

i) Total neurite length from all cells;

ii) Total number of neurite branches from all cells;

iii) Number of neurites per cell;

iv) Number of neurites per positive neuron;

v) Neurite length from each cell;

vi) Neurite length per positive neuron;

vii) Neurite length per neurite;

viii) Number of cells that are positive for neurite outgrowth;

ix) Percentage of cells positive for neurite outgrowth;

x) Number of branches per neuron; and

xi) Number of branches per neurite.

Based on all of the above, the combination of references cited by the Examiner clearly do not meet the requirements for establishing a prima facie case of obviousness of currently pending claim 19 under 35 USC 103(a). Pending claims 20-30 add further limitations to claim 19, and

thus are also not made obvious over the cited references. For example, none of the references, either alone or in combination, teach or suggest the limitations of pending claims 20-22, which recite the following:

20. The method of claim 19 wherein identifying cell bodies comprises the steps of:

generating a kernel image from the nuclear image;

performing conditional dilations of the kernel image to identify the cell body.

21. The method of claim 20, wherein identifying neurites extending from cell bodies comprises the steps of:

generating a reservoir image from the neurite image; and

identifying positive pixels in the reservoir image that are not present in the cell bodies, wherein such positive pixels belong to neurites extending from cell bodies.

22. The method of claim 21, further comprising

performing one conditional dilation of the kernel image to acquire a dilation image;

determining a set of nodes from the dilation image;

linking together connected nodes; and

repeating steps (A)-(C) until an entire neurite length has been traced.

Therefore, the cited references clearly do not meet the requirements for a prima facie case of obviousness as applied to any of the pending claims. Therefore, the Applicants respectfully request reconsideration and withdrawal of this objection.

Based on all of the above, the Applicants believe the claims are now allowable. If there are any questions or comments regarding this response, the Examiner is encouraged to contact the undersigned agent as indicated below.

Respectfully submitted,

Date: 12/17/01

David S. Harper
Registration No. 42,636

McDonnell Boehnen
Hulbert & Berghoff, Ltd.
300 South Wacker Drive
Chicago, IL 60606

Telephone: 312-913-0001
Facsimile: 312-913-0002

MARKED UP VERSION OF THE AMENDMENTS

In the specification:

Please replace the text at page 1 lines 4-15 with the following:

Please replace the text at page 16 lines 18-25 with the following:

Figure 4 illustrates an alternative embodiment of the invention in which cell arrays are in microwells 40 on a microplate 41, described [~~ion~~] **in** co-pending U.S. **Patent 6,103,479** [~~Application S/N 08/865,341~~], incorporated by reference herein in its entirety. Typically the microplate is 20 mm by 30 mm as compared to a standard 96 well microtiter plate which is 86 mm by 129 mm. The higher density array of cells on a microplate allows the microplate to be imaged at a low resolution of a few microns per pixel for high throughput and particular locations on the microplate to be imaged at a higher resolution of less than 0.5 microns per pixel. These two resolution modes help to improve the overall throughput of the system.

Please replace the text at page 20 lines 1-18 with the following:

Cell Arrays

Screening large numbers of compounds for activity with respect to a particular biological function requires preparing arrays of cells for parallel handling of cells and reagents. Standard 96 well microtiter plates which are 86 mm by 129 mm, with 6mm diameter wells on a 9mm pitch, are used for compatibility with current automated loading and robotic handling systems. The microplate is typically 20 mm by 30 mm, with cell locations that are 100-200 microns in dimension on a pitch of about 500 microns. Methods for making microplates are described in U.S. Patent **6,103,479** [~~App., Serial No. 08,865,341~~], incorporated by reference herein in its entirety. Microplates may consist of coplanar layers of materials to which cells adhere, patterned with materials to which cells will not adhere, or etched 3-dimensional surfaces of similarly pattered materials. For the purpose of the following discussion, the terms 'well' and 'microwell' refer to a location in an array of any construction to which cells adhere and within which the cells are imaged. Microplates may also include fluid delivery channels in the spaces between the wells. The smaller format of a microplate increases the overall efficiency of the system by minimizing the quantities of the reagents, storage and handling during preparation and the overall movement required for the scanning operation. In addition, the whole area of the

microplate can be imaged more efficiently, allowing a second mode of operation for the microplate reader as described later in this document.

Please replace the text at page 56 lines 4-23 with the following:

Cell preparation. The cells chosen for this study were mouse connective tissue fibroblasts (L-929; ATCC CCL-1) and a highly invasive glioblastoma cell line (SNB-19; ATCC CRL-2219) (Welch et al., *In Vitro Cell. Dev. Biol.* 31:610, 1995). The day before treatment with an apoptosis inducing drug, 3500 cells were placed into each well of a 96-well plate and incubated overnight at 37°C in a humidified 5% CO_2 atmosphere. The following day, the culture medium was removed from each well and replaced with fresh medium containing various concentrations of paclitaxel (0 – 50 μM) from a 20 mM stock made in DMSO. The maximal concentration of DMSO used in these experiments was 0.25%. The cells were then incubated for 26 h as above. At the end of the paclitaxel treatment period, each well received fresh medium containing 750 nM **MITOTRACKER®** Red (Molecular Probes; Eugene, OR) and 3 μg/ml Hoechst 33342 DNA-binding dye (Molecular Probes) and was incubated as above for 20 min. Each well on the plate was then washed with HBSS and fixed with 3.7% formaldehyde in HBSS for 15 min at room temperature. The formaldehyde was washed out with HBSS and the cells were permeabilized for 90 s with 0.5% (v/v) Triton X-100, washed with HBSS, incubated with 2 U ml^{-1} **BODIPY®** FL phallacidin (Molecular Probes) for 30 min, and washed with HBSS. The wells on the plate were then filled with 200 μl HBSS, sealed, and the plate stored at 4°C if necessary. The fluorescence signals from plates stored this way were stable for at least two weeks after preparation. As in the nuclear translocation assay, fluorescence reagents can be designed to convert this assay into a live cell high-content screen.

Please replace the text at page 56 lines 24-28 with the following:

Image acquisition and analysis on the ArrayScan System. The fluorescence intensity of intracellular **MITOTRACKER®** Red, Hoechst 33342, and **BODIPY®** FL phallacidin was measured with the cell screening system as described *supra*. Morphometric data from each pair of images obtained from each well was also obtained to detect each object in the image field (*e.g.*, cells and nuclei), and to calculate its size, shape, and integrated intensity.

Please replace the text at page 57 lines 1-19 with the following:

Calculations and output. A total of 50-250 cells were measured per image field. For each field of cells, the following calculations were performed: (1) The average nuclear area (μm^2) was calculated by dividing the total nuclear area in a field by the number of nuclei detected. (2) The average nuclear perimeter (μm) was calculated by dividing the sum of the perimeters of all nuclei in a field by the number of nuclei detected in that field. Highly convoluted apoptotic nuclei had the largest nuclear perimeter values. (3) The average nuclear brightness was calculated by dividing the integrated intensity of the entire field of nuclei by the number of nuclei in that field. An increase in nuclear brightness was correlated with increased DNA content. (4) The average cellular brightness was calculated by dividing the integrated intensity of an entire field of cells stained with **MITOTRACKER®** dye by the number of nuclei in that field. Because the amount of **MITOTRACKER®** dye that accumulates within the mitochondria is proportional to the mitochondrial potential, an increase in the average cell brightness is consistent with an increase in mitochondrial potential. (5) The average cellular brightness was also calculated by dividing the integrated intensity of an entire field of cells stained with **BODIPY®** FL phallacidin dye by the number of nuclei in that field. Because the phallotoxins bind with high affinity to the polymerized form of actin, the amount of **BODIPY®** FL phallacidin dye that accumulates within the cell is proportional to actin polymerization state. An increase in the average cell brightness is consistent with an increase in actin polymerization.

Please replace the text at page 95 lines 21-28 with the following:

In one embodiment, small reactive fluorescent molecules are introduced into living cells. These membrane-permeant molecules both diffuse through and react with protein components in the plasma membrane. Dye molecules react with intracellular molecules to both increase the fluorescence signal emitted from each molecule and to entrap the fluorescent dye within living cells. These molecules include reactive chloromethyl derivatives of aminocoumarins, hydroxycoumarins, eosin diacetate, fluorescein diacetate, some **BODIPY®** dye derivatives, and tetramethylrhodamine. The reactivity of these dyes toward macromolecules includes free primary amino groups and free sulfhydryl groups.

Please replace the text at page 96 lines 14-19 with the following:

In a second embodiment subdomains of the plasma membrane, the extracellular surface, the lipid bilayer, and the intracellular surface can be labeled separately and used as components of high content screens. In the first embodiment, the extracellular surface is labeled using a brief

treatment with a reactive fluorescent molecule such as the succinimidyl ester or iodoacetamde derivatives of fluorescent dyes such as the fluoresceins, rhodamines, cyanines, and **BODIPYS®**.

Please replace the text at page 97 lines 23-28 with the following:

Endosome fluorescence labeling

In one embodiment, ligands that are transported into cells by receptor-mediated endocytosis are used to trace the dynamics of endosomal organelles. Examples of labeled ligands include **BODIPY®** FL-labeled low density lipoprotein complexes, tetramethylrhodamine transferrin analogs, and fluorescently labeled epidermal growth factor (Molecular Probes, Inc.)

Please replace the text at page 98 lines 10-15 with the following:

Lysosome labeling

In one embodiment, membrane permeant lysosome-specific luminescent reagents are used to label the lysosomal compartment of living and fixed cells. These reagents include the luminescent molecules neutral red, N-(3-((2,4-dinitrophenyl)amino)propyl)-N-(3-aminopropyl)methylamine, and the **LYSOTRACKER®** probes which report intralysosomal pH as well as the dynamic distribution of lysosomes (Molecular Probes, Inc.)

Please replace the text at page 101 lines 10-14 with the following:

Mitochondrial labeling

In one embodiment, membrane permeant mitochondrial-specific luminescent reagents (Molecular Probes, Inc.) are used to label the mitochondria of living and fixed cells. These reagents include rhodamine 123, tetramethyl rosamine, JC-1, and the **MITOTRACKER®** reactive dyes.

In the claims:
Please cancel claim 18

~~1. An automated method for analyzing neurite outgrowth comprising~~

~~providing an array of locations comprising cells, wherein the cells possess at least a first luminescently labeled reporter molecule that reports on cell location, and at least a second luminescently labeled reporter molecule that reports on neurite outgrowth, and wherein the cells comprise neurons;~~

~~imaging or scanning multiple cells in each of the locations containing multiple cells to obtain luminescent signals from the first and second luminescently-labeled reporter molecule;~~

~~converting the luminescent signals into digital data; and~~

~~utilizing the digital data to automatically make measurements, wherein the measurements are used to automatically calculate changes in the distribution, environment or activity of the first and second luminescently labeled reporter molecules on or within the cells, wherein the calculated changes provide a measure of neurite outgrowth from the neurons.~~

~~18. A computer readable storage medium comprising a program containing a set of instructions for causing a cell screening system to execute the method of claim 1 wherein the cell screening system comprises an optical system with a stage adapted for holding a plate containing cells, a means for moving the stage or the optical system, a digital camera, a means for directing light emitted from the cells to the digital camera, and a computer means for receiving and processing the digital data from the digital camera.~~

Please add the following new claims

19. (NEW) An automated method for analyzing neurite outgrowth comprising

a) providing an array of locations comprising cells, wherein the cells possess at least a first luminescently labeled reporter molecule that reports on cell location, and at least a second luminescently labeled reporter molecule that reports on neurite outgrowth;

b) obtaining a nuclear image from the at least first luminescently labeled reporter molecule and a neurite image from the at least second luminescently-labeled reporter molecule;

c) automatically identifying cell bodies from the nuclear image;

d) automatically identifying neurites extending from the cell bodies from the neurite image; and

e) automatically determining one or more neurite features selected from the group consisting of:

i) Total neurite length from all cells;

ii) Total number of neurite branches from all cells;

iii) Number of neurites per cell;

iv) Number of neurites per positive neuron;

v) Neurite length from each cell;

vi) Neurite length per positive neuron;

vii) Neurite length per neurite;

viii) Number of cells that are positive for neurite outgrowth;

ix) Percentage of cells positive for neurite outgrowth;

x) Number of branches per neuron; and

xi) Number of branches per neurite.

20. (NEW) The method of claim 19 wherein identifying cell bodies comprises the steps of:

A) generating a kernel image from the nuclear image;

B) performing conditional dilations of the kernel image to identify the cell body.

21. (NEW) The method of claim 20, wherein identifying neurites extending from cell bodies comprises the steps of:

I) generating a reservoir image from the neurite image; and

II) identifying positive pixels in the reservoir image that are not present in the cell bodies, wherein such positive pixels belong to neurites extending from cell bodies.

22. (NEW) The method of claim 21, further comprising

(a) performing one conditional dilation of the kernel image to acquire a dilation image;

(b) determining a set of nodes from the dilation image;

(c) linking together connected nodes; and

(d) repeating steps (a)-(c) until an entire neurite length has been traced.

23. (NEW) The method of claim 22, wherein steps (a) through (d) are carried out at multiple time points.

24. (NEW) The method of claim 19 further comprising contacting the neurons with a test compound, and determining an effect of the test compound on neurite outgrowth from the cell bodies.

25. (NEW) The method of claim 24, further comprising contacting the neurons with a neurotoxin either before, after, or simultaneously with the test compound.

26. (NEW) The method of claim 24, further comprising contacting the cells with a control compound known to stimulate neurite outgrowth, and determining whether the test compound inhibits the control compound from inducing neurite outgrowth from the cell bodies.

27. (NEW) The method of claim 19, wherein steps b) through e) are carried out at multiple time points.

28. (NEW) The method of claim 19 wherein the first luminescently labeled reporter molecule comprises a DNA binding compound.

29. (NEW) The method of claim 19 wherein the second luminescently labeled reporter molecule is neuron-specific.

30. (NEW) The method of claim 29 wherein the neuron-specific luminescent reporter molecule comprises a molecule selected from the group consisting of neurofilament proteins, βIII-tubulin, ciliary neurotrophic factor, and antibodies specific for neurofilament proteins.